Research Journal of Fisheries and Hydrobiology, 1(1): 23-27, 2006

Effects of Temperature on the Incubation Period and Reproductive Performance of Berried Female Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758) Under Cultured Conditions

[1,3]Efrizal, [1]A. Arshad, [2]M.S. Kamarudin and [2]C.R. Saad

[1]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang Selangor Darul Ehsan, Malaysia
[2]Department of Agrotecnology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang Selangor Darul Ehsan, Malaysia
[3]Faculty of Fisheries, Bung Hatta University, Jln. Sumatera Ulak Karang, Padang, 25133, West Sumatera, Indonesia

Abstract: This study was carried out to investigate the effects of different temperature on incubation period, and reproductive performance of berried female blue swimming crab, *Portunus pelagicus*. The results showed that the temperatures significantly affect the incubation period, fertilization and hatching rate of egg. The egg incubation period decreased exponentially from 8.33 to 6.67 days with increasing temperature in the range 28-34°C. The best fertilization and hatching rate of eggs were obtained at temperature in the range 28-30°C and 28-32°C, respectively. Relationship between temperature and incubation period, fertilization and hatching rate of eggs was found to be quadratic.

Key words: *Portunus pelagicus*, blue swimming crab, temperature, incubation period, reproductive performance.

INTRODUCTION

Estimates of duration time of the incubation period, especially in the case of edible species, are among the most important information concerning hatching and spawning process. This information is required prior to establishment of a good hatchery management program, which would ensure the sustainable production of larvae and juveniles of cultured species.

Most works on incubation period and reproductive performance have been established on marine and freshwater fish species, and limited studies have been conducted on crabs, mainly on commercially exploited species such us *S. serrata* and *P. pelagicus.* Incubation in other crustacean groups such as the barnacles[26], the copepods[4] and brachyuran[33,21,15,] have been studied in relation to temperature. Temperature is also a variable that is known to affect the rate of egg development[16,3,13] larval survival rate and development in many brachyurans[22,34,23,13,]. However, little is known about temperature effects on incubation period and reproductive performance of individual blue swimming crab species. The objective of the present study was to determine the effect of temperature on incubation period and reproductive performance of berried female blue swimming crab, *P. pelagicus,* under cultured conditions.

MATERIALS AND METHODS

Twelve ovigerious crabs (body weight of 126.4 to 268.4 g and carapace width of 106.39 to 140.58 mm) were held in 125 liter aquaria at density of 1 individual/ 75 liter with gentle aeration. Females were not fed during the incubation and hatching period. Different temperature levels (28, 30, 32, and 34°C) were designated as treatments A, B, C and D with each temperature level replicated three times. Temperatures in the aquaria were controlled using thermoregulated electric heaters. Salinity (30 ppt) and temperature measurements were performed with a direct reading digital salinometer (YSI model 58 oxygen meter, Yellow Springs Instrument Co., Yellow Springs, OH, USA).

Stage of embryonic development was determined by examining eggs that were periodically removed from the crab. The egg incubation period was determined as the number of days from spawning to the hatching days. Samplings for egg fertilization rate were conducted on the 6th and 8th day. Egg samples were taken from different points of the egg mass. Fertilized eggs were pigmented, irregular in shape and manifested eye formation while unfertilized eggs were unpigmented, spherical and uniformly dark or a black mass. Number of eggs counted

Corresponding Author: Efrizal C/O. Dr Aziz Arshad, Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang Selangor Darul Ehsan, Malaysia
E-Mail: efrizal_ubh@yahoo.com

ranged from 500 to 700. Percentage fertilization rate of eggs was computed as follows[21].

$$FR = \frac{FE}{FE + UFE} \times 100\%$$

Where: FR = Fertilization rate (%)
FE = Fertilized eggs
UFE = Unfertilized eggs

Upon hatching, the total number of zoea larvae produced was estimated from six, 50 ml aliquot water samples taken from the hatching tank[21]. The newly hatched zoea, and unfertilized eggs were counted from the sample and hatching rate was calculated using the following formula:

$$HR = \frac{HZ}{TNE(HZ + UFE)} \times 100\%$$

Where: HR = Hatching rate (%)
HZ = Hatched zoea
TNE = Total number of eggs
UFE = Unfertilized eggs

The data of incubation period (days) and reproductive performance (%, egg fertilization and zoeal hatching rate) were tested using one way ANOVA and Duncan's Multiple Range test was used to compare the differences among treatments[30]. Arcsin transformation was done in the analysis of the data in percentage.

The relation ship between temperature (t) and incubation period (IP), fertilization rate (FR) and hatching rate (HR) were analysed using statistical computer software, SPSS version13.0, for significant correlation between them

RESULTS AND DISCUSSIONS

Incubation Period: Data (Table 1) showed that incubation period had significantly been ($p<0.05$) affected by the temperature level. The highest temperature (34°C) resulted in the shortest ($p<0.05$) incubation period (6.67±0.41 days). Regression analysis found the relationship to be quadratic ($IP = -0.0417t^2 + 2.3167t–23.9$; $R^2 = 0.6900$; $p<0.05$; Fig. 1).

This study showed that the temperature significantly affected the incubation period of *P. pelagicus* eggs. According to Wear[33], Heasman and Fielder[16], Choy[3], Zeng *et al.*[35] and Hamasaki[13], temperature is one of the most important factors in regulating egg development for several brachyuran species including *Scylla paramamosain*[14]. In a similar study, the lower temperature slow down the incubation period of 10 newly berried *Palaemon serratus* females[28]. He tested three different temperatures (10, 15 and 20°C) and found that 20°C gives the fastest incubation period (28 days), whereas at 10°C and 15°C, the incubation period is

Table 1: Mean±s.e. Incubation period of berried female blue swimming crab, *P. pelagicus* at different temperatures. Means within a given column with different superscripts were significantly different ($p<0.05$).

Treatment (n =3)	Incubation period (days)
A (28°C)	8.33±0.41^a
B (30°C)	8.00±0.00^a
C (32°C)	7.67±0.41^a
D (34°C)	6.67±0.41^b

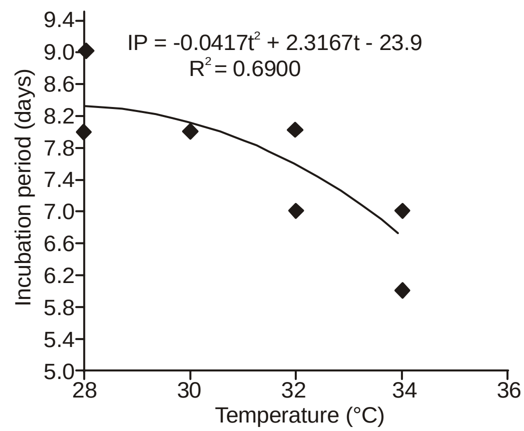

Fig. 1: Relationship between mean temperature (t) and incubation period (IP) of berried female blue swimming crab, *P. pelagicus*.

prolonged to 95 and 58 days, respectively. Heasman and Fielder[16] reported that duration of successful incubations for *S. serrata* varies from 20 to 30 days where the shortest incubation period is achieved at inversely rising temperature. In this study, the egg incubation period of *P. pelagicus* lasted for 6.67-8.33 days; it decreased exponentially with increasing temperature in the range between 28°C-34°C. A similar pattern was observed by Wear[33] and Hamasaki[15] with British decapod crustacean and *S. serrata*, respectively.

The relationship between temperature and the egg incubation period from spawning to hatching in *S. paramamosain* and the biological zero and threshold temperature were estimated as 12.19 and 13.98°C, respectively[14]. Heasman and Fielder[16] and Zeng *et al.*[35] applied Bělehrádek's equation to the relationship between temperature and egg incubation period from spawning to hatching for the mud crab species as *S. serrata*. Parameters of the equation estimated by Zeng *et al.*[35] are similar to those estimated by Hamasaki[14] for *S. paramamosain*. On the other hand, the equation curved proposed by Heasman and Fielder[16] shows a different locus from both *S. serrata*[15] and *S. paramamosain*[14]. The mud crab species based on current nomenclature[17] examined by Heasman and Fielder[16] is not clear, but the crab used by Zeng *et al.*[35] is almost certainly *S. paramamosain* according to Li *et al.*[18]. The lower temperature merely slow down the rate of egg development, and the present study did not reach the lowest limits of egg tolerance.

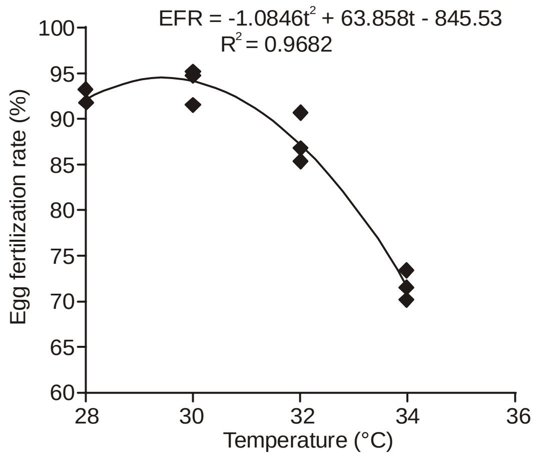

Fig. 2: Relationship between mean temperature (t) and egg fertilization rate (EFR) of berried female blue swimming crab, *P. pelagicus*.

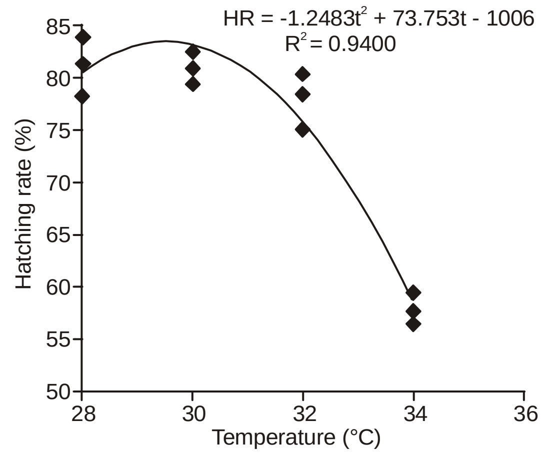

Fig. 3: Relationship between mean temperature (t) and hatching rate (HR) of berried female blue swimming crab, *P. pelagicus*.

Table 2: Mean±s.e. Egg fertilization rate (%) of berried female blue swimming crab, *P. pelagicus* incubated at different temperatures. Means within a given column with different superscripts were significantly different ($p<0.05$).

Treatment (n=3)	Number of eggs Fertilized	Total	Fertilization rate (%)
A (28 °C)	662.67±5.31	718.00±1.87	92.29±0.57[a]
B (30 °C)	669.00±2.57	713.33±3.19	93.78±1.38[a]
C (32 °C)	625.33±2.46	713.67±1.78	87.63±1.96[b]
D (34°C)	513.33±6.42	715.33±2.16	71.77±1.11[c]

Fertilization Rate: The percentage of successful fertilization rate at different temperature level was generally high ranging from 71.77-93.78%. The fertilization rate (%) decreased with increasing temperature ($p<0.05$). The highest fertilization rate was achieved at 28°C and 32°C, whereas the lowest ($p<0.05$) fertilization rate occurred at 34°C with 71.77±1.11 % (Table 2). The relationship between temperature and egg fertilization rate was quadratic with an equation of $EFR = -1.0846t^2 + 63.858t - 845.53$ (Fig. 2). This relationship was found to be highly significant ($R^2 = 0.9682$; $p<0.05$)

Temperature exerts a strong effect on marine teleost eggs[1,2] and embryonic[7,33,9,10] and larval development[29,19,27] of decapod crustaceans. At tolerable levels, temperature controls metabolic and development rates[8]. Extreme levels of temperature can result in mortality during egg incubation or cause developmental anomalies that reduce fertilization rate and larval viability. In this study, the optimum temperature of egg fertilization rate of blue swimming crab was in the range of 28 to 30°C and the lowest fertilization rate occurred at high temperature of 32 to 34°C. Temperature ≥ 32°C seemed to inhibit the egg growth and development.

Effects of temperature on fertilization rate of decapod crustacean eggs are poorly known. However, embryonic development duration under different temperatures are described in several studies. Garcia-Guerrero and

Table 3: Mean±s.e. Zoeal hatching rate (%) of berried female blue swimming crab, *P. pelagicus* at different temperatures. Means within a given column with different superscripts are significantly different ($p<0.05$).

Treatment (n=3)	Number of eggs Zoael hatching	Unfertilized	Total	Hatching rate (%)
A (28 °C)	530,545.75± 107,204.10	126,211.85± 37,889.78	656,757.60± 142,504.60	81.09±1.96[a]
B (30 °C)	552,342.82± 194,240.51	127,755.1± 38,002.04	680,097.93± 327,815.54	80.90±1.02[a]
C (32°C)	509,519.02± 229,278.47	136,294.77± 47,828.73	645,813.79± 277,015.56	77.95±1.89[a]
D (34 °C)	361,370.24± 58,219.73	266,731.25± 53,264.17	628,101.49± 111,454.89	57.79±1.07[b]

Hendrikx[11] determined the embryonic development from recently spawned eggs to hatching lasts 14 days for *Aratus pisoni* and 15 days for *Goniopsis pulchra* at 26-28°C. Reported that the embryonic development of mangrove crab, *Ucides cordatus*, takes 19±1 day at 27°C.

Hatching Rate: Temperature (28, 30, 32 and 34°C) significantly influenced ($p<0.05$) hatching rate (Table 3). Similar to fertilization rate, the highest zoeal hatching rate of *P. pelagicus* eggs was achieved at lower temperatures (28-32°C), whereas the lowest hatching rate of eggs was found at 34°C (57.79±1.07 %). Statistical analysis using ANOVA with DMRT test demonstrated that zoeal hatching rate reared at 34 % was significantly lower than those at other temperature. The relationship between zoeal hatching rate and temperature was found to be quadratic ($HR= -1.2483t^2 + 73.753t - 1006.9$; $R^2 = 0.9400$; $p<0.05$; Fig. 3).

In series of studies on the hatching process in aquatic invertebrates, Davis[6] found that the egg size decapod crustaceans increases during incubation due to either a slow but steady osmotic swelling of the inner egg membrane or to swelling of the embryo itself. In both cases, Davis considered that the size increase is brought about by uptake of water which increases the internal pressure of the egg up to the time hatching.

Some environmental factors related to hatching success have been examined. Gillet[12], experimenting with landlocked Swiss Arctic char, reported that survival rate of eyed eggs is slightly better when parents collected from the field are maintained at 5ºC than when they were kept at 8ºC. Egg survival is also higher if females are collected later in the season. Steiner[31] reported that the hatching of landlocked Austrian Arctic char eggs is slightly higher at 5 and 7ºC (about 90 %) than at 3 and 8ºC (about 85 %).

Regretfully, the effect of temperature to hatching rate on tropical marine crab eggs is poorly known. In the present study, the optimum temperature for best hatching rate of egg blue swimming crab, *P. pelagicus,* was found at the range of 28 to 32ºC with mean of 77.95 to 81.09 %, respectively. Higher temperature (34ºC) seemed to inhibit the egg growth and development, while the number of hatched zoea decreased markly (57.79 %). Consequently, a high zoeal mortality was related with the low fertilization rate at 34ºC. Watanabe[32] reported that hatching success of *Epinephelus striatus* eggs is high (avg. = 82.5 %) at all temperatures within the range of 26-30ºC, but mortality of yolksac larvae is accelerated at high temperature.

The result of this study also demonstrated that hatching of *P. pelagicus* eggs was improved if eggs were incubated at low temperature. It is not known whether this was related to advanced development at fertilization rate. A similar observation was also reported and described by Lucyzynski[20] for vendace (*Coregonus albula*). In her study, low temperatures used only at late stages to delay hatching; the juveniles had little or no yolk at hatching and needed to feed. The effects of these temperatures on early survivorship, given adequate food were not reported. Survival after swim-up may be improved by warm temperatures, which young char may choose in the wild.

Research has indicted that incubation temperature affects the rate of embryonic and larval development from zoeal stages through to early crab stages[24,25,33,16,15]. Increasing water temperature during the incubation period accelerates the rate of embryonic development in crabs[33,16,15]. Consequently by manipulating water temperature, crab culturists can control the time of hatching. Although temperature manipulation is an attractive management option[5], there is evidence that shortening the incubation period by increasing the temperature will significantly affect the fertilization rate and hatching rate of the blue swimming crab, *P. pelagicus*. According to Wear[33] rising water temperature to achieve rapid incubation period can disrupt the normal interval between the spawning of successive egg batch and cause significant reduction to the egg membrane.

ACKNOWLEDGEMENTS

I am grateful to Head of the Department of Biology, Faculty Science, Universiti Putra Malaysia for providing necessary of laboratory facilities. Financial support by University of Bung Hatta, Padang, West Sumatera is gratefully acknowledged.

REFERENCES

1. Blaxter, J.H.S., 1969. Development: eggs and larvae. In: W.S. Hoar and D.J. Randall (Eds.), Fish Physiology, Vol. 3. Academic Press, New York, NY, pp: 177-252.
2. Blaxter, J.H.S., 1988. Pattern and variety in development. In: W.S. Hoar and D.J. Randall (Editors), Fish Physiology, Vol. 11, Part A. Academic Press, San Diego, CA, pp: l-58.
3. Choy, S.C., 1991. Embryonic and larval biology of *Liocarcinus holsatus* and *Necora puber* (Crustacea: Brachyura: Portunidae). J. Exp. Mar. Biol. Ecol. 148, pp: 77-92.
4. Corkett, C.J., and I.A. McLaren, 1970. Relationships between development rate of eggs and older stages of copepods. Journal of Marine Biological Sciences and and the Biological Station, 22, 1-5.
5. Crisp, D.T., 1988. Prediction, from temperature, of eyeing, hatching and 'swimp-up times of salmonid embryos. Freshwater Biology, 41-48.
6. Davis, C.C., 1966. A study of the hatching process in aquatic invertebrates. XXIII. Exclosin in *Petrolisthes armatus* (Gisbbes) (Anomura, Porcellanidae). Internationale Reveu der gesamtem Hydrobiologie u Hydrographis. 51: 6-791.
7. Efford, I.E., 1969. Egg size in sand crab, *Emerita analoga* (Decapoda, Hippidae).-Crustacean 16: 15-26.
8. Fry, F&J., 1971. The effect of environmental factors on the physiology of fish. In: Hoar W.S. and D.J. Randall (Editors)) Fish Physiology, Vol, 6. Academic Press, New York, NY, pp: l-98.
9. Fukui, Y., 1988. Comparative studies on the life history of the grapsid crabs (Crustacea, Brachyura) inhabiting intertidal cobble and boulder shores. Publication of the Seto Marine Biology Laboratory 33: 121-162.
10. Furota, T., 1988. The ecology of introduced spider crab *Pyromaia tuberculata* in an organically polluted bay (Preliminary Report). Benthos Research 33/34: 79-89.
11. García-Guerrero, M. and M.E. Hendrickx, 2004. Embryology of decapod crustaceans I. Embryonic development of the mangrove crabs *Goniopsis pulchra* and *Aratus pisonii* (Decapoda: Brachyura). Journal of Crustacean Biology: Vol. 24, No. 4, pp: 666-672.

12. Gillet, C., 1991. Egg production in an Arctic charr (*Salvelinus alpinus* L.) brood stock: effects of temperature of the timing of spawning and quality of eggs, Aquatic Living Resources, 4: 109-116.
13. Hamasaki, K., 1996. Study on the reproduction and development of the swimming crab, *Portunus trituberculatus*. Special Scientific Report of Japan Sea-Farming Association 8. 124 pp. (in Japanese with English summary).
14. Hamasaki, K., 2002. Effects of temperature on the survival, spawning and egg incubation period of overwintering mud crab broodstock, *Scylla paramamosain* (Brachyura: Portunidae). *Suisanzoshoku* 50, pp: 301-308.
15. Hamasaki, K., 2003. Effects of temperature on the egg incubation period, survival and developmental period of larvae of the mud crab *Scylla serrata* (Forskål) (Brachyura: Portunidae) reared in the laboratory. Aquaculture 219, pp: 561-572.
16. Heasman, M.P. and D.R. Filder, 1983. Laboratory spawning and mass rearing of the mangrove crab, *Scylla serrata* (Forskal), from first zoea to first crab stage. Aquaculture, 34: 303-316.
17. Keenan, C.P., P.J.F. Davie and D.L. Mann, 1998. A revision of the genus *Scylla* de Hann, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). Raffles Bull. Zool. 46, pp: 217-245.
18. Li, S., C. Zeng, H. Tang, G. Wang and Q. Lin, 1999. Investigations into the reproductive and larval culture biology of the mud crab, *Scylla paramamosain*: a research overview. In: Keenan, C.P. and Blackshaw, A., Editors, 1999. Mud Crab Aquaculture and BiologyACIAR Proceedings vol. 78, pp: 121-124.
19. Lindley, J.A., 1990. Regressions for estimating development in the shrimp, *Palaemonetes pugio* Holthuis (Caridea, Palaemonidae). Crustacean (Suppl. 2): 19-26.
20. Luczynski, M., 1986. "Hunger potential" of *Coregonus albula* L. Larvae of different hatching time. Comperative Biochemistry and Physiology, 84A: 459-460.
21. Millamena, O.M. and E. Quinitio, 2000. The effects of diets on reproductive performance of eyestalk ablated and intact mud crab *Scylla serrata.* Aquaculture, 181, pp: 81-90.
22. Minagawa, M., 1990. Influence of temperature on survival, feeding and development of larvae of the red frog crab, *Ranina ranina* (Crustacea, Decapoda, Raninidae). Nippon Suisan Gakkaishi 56, pp: 755-760.
23. Okamoto, K., 1993. Influence of temperature on survival and growth of larvae of the giant spider crab *Macrocheira kaempferi* (Crustacea, Decapoda, Majidae). Nippon Suisan Gakkaishi 59, pp: 419-424.
24. Ong, K.S., 1964. The early developmental stages of *Scylla* serrata (Forskall) (Crustacea, Portunidae), reared in the laboratory. Proc. Indo-Pacific Fish. Counc., 11 (2): 135-146.
25. Ong, K.S., 1966. Observations on the post-larval life history of *Scylla serrata* Forskall, reared in the laboratory. The Malaysian Agriculture Journal, Vol. 45 No. 4, pp: 429-443.
26. Patel, B. and D.J. Crisp, 1960. Rates of development of the embryos of seven several of barnacles. Physiological Zoology, 33: 19-104.
27. Penheiro, M.A.A. and M.L. Negreiros-Fransozo., 1994. Estimative da duracão larval em funcã da temperature para a Família Majidae (Crustacea, Decapoda, Brachyura). Boletim do Instituto de Pesca, 21: 75-81.
28. Reeve, M.R., 1969. The laboratory culture of the prawn Paleomon serratus. Fishery Investigations, Ministry of Agriculture, Fisheries and Food, Ser. 2, 26: 1-38.
29. Sastry, A.N., 1983. Ecological aspects of reproduction. In D.E. Bliss, ed,-in-chief, The Biology of Crustacea. Vol. 5. F.J. Vemberg and W.B. Vemberg, eds. Enviromental Adaptations. Academic Press, New York, New York. pp: 179-270.
30. Steel, R.D.G. and J.H. Torrie, 1980. *Principles and procedures statistics*. *A biometrical approach*. MCGraw-Hill, New York, NY. pp: 633.
31. Steiner, V., 1984. Experiments toward improving the culture of Arctic charr (*Salvelinus alpinus* L.). Pages 509-521 in L. Johnson and B.L. Burns, editors. Biology of the Arctic charr. University Manitoba Press, Arctic charr (*Salvelinus alpinus* L.) Winnipeg.
32. Watanabe, W.O., L. Cheng-Sheng, S.C. Ellis and E.P. Ellis, 1995. Hatchery study of the effect of temperature on eggs and yolksac larvae of the Nassau grouper *Epinephelus striatus*. Aquaculture, 136: 141-147.
33. Wear, R.G., 1974. Incubation in British decapoda crustaceans and the effects of temperature on the rate and success of embryonic development. J. Mar. Biol. Assoc. UK 54, pp: 745-762.
34. Zeng, C. and S. Li, 1992. Effects of temperature on survival and development of the larvae of *Scylla serrata*. J. Fish. China 16, (in Chinese with English abstract). pp: 213-221.
35. Zeng, C., G. Wang and S. Li, 1991. Observations on embryonic development and effects of temperature on developmental rate of embryonic stages in mud crab, *Scylla serrata*. Fujian Fisheries 1991 1, (in Chinese). pp: 45-50.